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Risk Assessment Branch VI, Health Effects Division (7509P) **Date:** _____

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DATA EVALUATION RECORD[1]

STUDY TYPE: Immunotoxicity [dietary] - Rat OPPTS 870.7800

PC CODE: 016331 **DP BARCODE:** D410187

TEST MATERIAL (PURITY): Momfluorothrin (95.7%)

SYNONYMS: S-1563

CITATIONs: Hosako H. (2012). S-1563- A 28-Day Oral (Dietary) Immunotoxicity Study in Male Wistar Han Rats. WIL Research, 1407 George Road, Ashland, OH 44805-8946. Project ID: WIL-118068. MRID# 49020048. Unpublished.

SPONSOR: Sumitomo Chemical Co. Ltd., 27-1, Shinkawa 2-chome, Chuo-ku, Tokyo 104-8260, Japan.

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID #49020048), S-1563 (95.7%, Lot No. 9CMO109G) was administered to male Crl:WI(Han) rats (10/dose) in the diet at dose levels of 0, 300, 1000 or 3000 ppm (equivalent to 0, 26, 81, and 241mg/kg bw/day, respectively) for 28 consecutive days. The positive control group (10 males) was administered 50 mg/kg bw/day (dose volume of 10 mL/kg b.w.) of cyclophosphamide monohydrate (CPS) intraperitoneal injection (IP) from Days 24 through 27. On study Day 24, all animals in all groups received a single intravenous (IV) dose of sheep red blood cells at 0.5 mL/animal (2×10^8 SRBC/animal). During the study, clinical condition, bodyweight, food and water consumption, organ weight, and macroscopic pathology were evaluated. At sacrifice (Day 29), selected organs were removed and weighed (spleen, thymus and adrenal gland). The anti-SRBC IgM response was measured with an antibody-forming cell (AFC) assay.

There were no premature deaths and no treatment-related clinical signs. There were no treatment- related effects on clinical observations, gross pathology, and organ weights. At 3000 ppm, there were treatment- related lower body weight gains and food consumption. Positive control group animals had decreased terminal body weights (11% less than vehicle control group) and statistically significantly ($p \leq 0.01$) decreased absolute and relative spleen and thymus weights.

1 Disclaimer: The attached Data Evaluation Record is a modified version of the Tier II Summary provided by Sumitomo Chemical Co. Ltd. Portions of this document may have been altered by the EPA reviewer.

The systemic LOAEL was 3000 ppm (equivalent to 241 mg/kg bw/day) based on lower body weight gains and food consumption. The systemic NOAEL was 1000 ppm (equivalent to 81 mg/kg bw/day).

There were no statistically significant differences in T-cell dependent antigen response (TDAR) to sheep red blood cells, either specific activity ($AFC/10^6$ spleen cells) or as total activity (AFC/spleen), between treated groups and the vehicle control group. High inter-individual variability was noted in all the treatment groups as well as in the control group. Evaluation of the individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of TDAR response to sheep RBCs. Positive control (CPS) group animals had statistically significantly ($p \leq 0.01$) decreased TDAR response. This confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for S-1563 does not reveal any evidence of immunotoxicity. The overall weight of evidence suggests that the chemical does not directly target the immune system.

The NOAEL for immunotoxicity was 3000 ppm (equivalent to 241 mg/kg bw/day), the highest dose tested. The immunotoxicity LOAEL was not established.

This immunotoxicity study is classified **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats. The lack of stability data was noted as a minor deficiency. However, this is not expected to impact the results of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

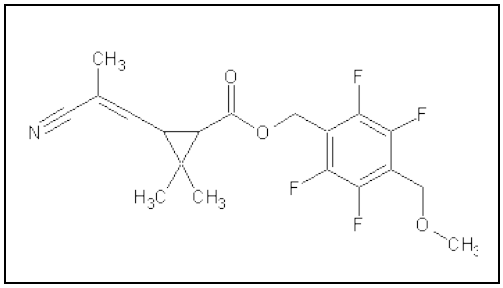
A. MATERIALS:

1. Test material:

S-1563

Description: Off-white, coarse powder
Lot/Batch #: 9CMO 109G
Purity: 95.7 %
Compound Stability: Stable at 2ºC to 10ºC; Expiratory. Date: 2 Feb. 2012
CAS # of TGAI: 609346-29-4

Structure



2. Vehicle and/or positive control:

Vehicle: No vehicle, mixed directly into the diet Certified Rodent LabDiet® 5002 (meal).
Positive Control: Cyclophosphamide Monohydrate; Lot no. 120M1253V, from Sigma-Aldrich, St. Louis, MO, USA.

3 Test animals:

Species: Rat, male
Strain: Crl:WI(Han)
Age/weight at treatment initiation : Approximately 7 weeks old/ 181-227 g.
Source: Charles River Lab., Raleigh, NC, USA
Housing: Housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board.
Diet: *Certified Rodent LabDiet® 5002 (meal), ad libitum*
Water: Reverse Osmosis treated water, *ad libitum*
Environmental conditions:
Temperature: 22±3 °C
Humidity: Approximately 50±20%
Air changes: 10/hour
Photoperiod: 12 hrs dark/ 12 hrs light
Acclimation period: **14 days**

B. STUDY DESIGN:

1. **In life dates** – Treatment Start: January 3, 2012 End: January 31, 2012.

2. **Animal assignment**: A computerized randomization procedure was used. Body weight variation did not exceed ± 20% of the mean weight.

Table 1. Study Design[a]			
Test group	**Conc. in diet (ppm)**	**Actual time-weighted average dose (mg/kg/day)**	**No. of male animals**
1. Vehicle Control	0	0	10
2. S-1563	300	26	10
3. S-1563	1000	81	10
4. S-1563	3000	241	10
5. Positive Control[b]	0	50[b]	10

[a] Information was obtained from page 25 of the study report
[b] Positive Control group received Cyclophosphamide IP. 50 mg/kg bw/day from Day 24 to 27.

3. **Dose selection:** The dose levels were selected based on the previously conducted dose range-finding study of S-1563 in male Wistar Han rats (Hosako, 2012, WIL-118067). There was a statistically significant reduction of body weight gain both at 3000 ppm and 6000 ppm.

4. **Diet preparation and analysis:** It was mentioned that the test substance was added to a portion of the diet and blended in a Hobart mixer. The resulting premix was then mixed thoroughly with the remaining feed in a Hobart mixer to obtain the appropriate dietary concentration.

Results

Homogeneity analysis:

	Group 2 (300 ppm)	Group 3 (1000 ppm)	Group 4 (3000 ppm)
Homogeneity Assessment of the 2 January 2012 Formulations			
Mean Concentration (ppm)	309	1038	3003
RSD (%)	1.9	1.4	1.7
Mean % of Target	103	104	100

Concentration analysis:

	Mean Concentration, ppm (% of Target)		
Date of Preparation	Group 2 (300 ppm)	Group 3 (1000 ppm)	Group 4 (3000 ppm)
2 January 2012	304 (101)	1027 (103)	3010 (100)

Stability: It was established in a previous study (10 days, WIL-118069).

5. **Statistics:** Bartlett's Chi Square test was used for homogeneity of variances. Homogeneous data were analyzed with parametric one-way ANOVA (Kruskal and Wallis, 1952). If significant, the test groups were compared with vehicle control group using Dunnett's test. Non- homogenous data were evaluated using a non-parametric ANOVA. If significant, test groups were compared to the vehicle control group using the Gehan-Wilcoxon test. The Jonckheere's test was used to analyze dose-related trends across the vehicle control and treatment groups. The positive control data was evaluated with Student's t-Test. The criteria for accepting the results of the positive control group included a statistically significant ($p \leq 0.5$) decrease in the response compared to that of the vehicle control group.

C. METHODS:

1. **Observations:** Animals were inspected at least twice a day for treatment- related health effects. Detailed physical examination was done once every week.

2. **Body weight:** Body weights were recorded on Day-0 and twice per week throughout the study period and before necropsy.

3. **Food/water consumption and compound intake:** During the test period, food intake was recorded weekly.

4. **Sacrifice and pathology:** On study Day 29, animals were sacrificed with carbon dioxide (CO_2) inhalation and exsanguination. Blood was collected via the inferior vena cava from all animals at the time of euthanasia.

   a. Gross necropsy: Following gross examination, spleen, thymus, and adrenal gland weights were recorded.

   b. Tissue preparation/histopathology: Not performed.

5. **Immunotoxicity:**

   a. Antibody-Forming Cell (AFC) assay: On study Day 24, all animals in all groups received a single intravenous dose of sheep red blood cells 0.5 mL (2×10^8 SRBC/mL) in the lateral tail vein. The positive control group (10 males) was administered 50 mg/kg bw/day of cyclophosphamide (IP) from Days 24-27. After sacrifice, S-1563 effect on immune response to a T-cell dependent immunogen (SRBC) was evaluated using the AFC assay (a modified Jerne plaque assay). Model Z1 Coulter Counter was used for spleen cell count. Propidium iodide and flow cytometry were used to determine viability of splenocytes.

   b. NK cell Assay: Did not perform NK cell assay.

II. RESULTS:

A. OBSERVATIONs:

1. Clinical signs of toxicity: No clinical signs of toxicity were observed.

2. Mortality: There were no unscheduled mortalities during the study.

B. Body weight and weight gain: At 3000 ppm, a slight (-7%) but not statistically significant decrease of body weight was observed; statistically significantly lower mean cumulative body weight gains were noted throughout the dosing period when compared to the vehicle control group. The mid dose group (1000 ppm) had transient slightly lower body weight gain from study Day 0 to 3 (Table 2 and 3). The positive control group had 11% decrease mean terminal body weight when compared to the vehicle control group.

TABLE 2. Mean body weights (g) ± SD

Study Day	0 ppm	300 ppm	1000 ppm	3000 ppm	Positive control
0	201.1±11.3	200.4±12.02	201.0±11.3	199.2±14.6	200.6±13.1
7	248.3±12.8	246.6±15.8	242.5±13.1	233.9±16.9	246.5±13.9
14	278.6±13.3	277.2±19.61	269.9±16.9	261.1±20.4	276.0±15.5
21	306.3±12.5	305.6±24.2	293.6±22.6	285.3±23.6	303.7±17.1
28	335.7±13.8	335.0±27.5	321.7±27.5	311.3±25.7	**299.7** ±23.8**

Data obtained from pages 53 - 63 in the study report.
* p<0.05; ** p≤ 0.01
Positive control group was given cyclophosphamide IP 50 mg/kg bw/day from Days 24-27.

Table 3. Summary of Cumulative Body Weight Changes (g) ± SD

Study Day	0 ppm	300 ppm	1000 ppm	3000 ppm	Positive Control
0-7	47.2±2.6	46.2±7.3	41.5±6.2	**34.7**±6.2**	45.9±5.2
0-14	77.5±5.4	76.8±12.7	68.9±12.3	**61.9**±10.9**	75.4±10.5
0-21	105.2±6.6	105.2±18.3	92.6±19.3	**86.1**±14.5**	103.1±13.4
0-28	134.6±6.9	134.6±23.2	120.7±25.02	**112.1**±16.9**	**99.1**±23.6**

Data obtained from pages 76-83 of the study report
* p≤ 0.05; ** p≤ 0.01
Positive control group was given cyclophosphamide IP 50 mg/kg bw/day from Days 24-27.

C. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE:

1. Food consumption/ Food Efficiency: At 3000 ppm significantly lower food consumption was noted from study Day 0 to 3 only. Thereafter, mean food consumption in this group remained slightly lower than the vehicle control group until the end of the dosing period.

2. Compound consumption: The compound consumption in each group was shown in Table 1.

D. GROSS NECROPSY: No treatment related visible lesions were found.

1. Organ weight: There were no statistically significant differences in organ weights between the treated groups and the vehicle control group. Positive control group animals (cyclophosphamide) had statistically significant ($p \leq 0.01$) decreases in absolute and relative spleen and thymus weights when compared to the vehicle control group (Table 4).

Table 4. Final Body Weights, Absolute and Relative Organ Weights, Mean Values

Parameter	Vehicle Control (10)	S-1563 (ppm) 300 (10)	1000 (10)	3000 (10)	CPS 50 mg/kg	H/NH	Trend Analysis
Body Wgt (g)	336 ± 4	335 ± 9	322 ± 9	311 ± 8	300 ± 8**	H	p ≤ 0.05
Spleen (mg)	769.4 ± 21.7	686.8 ± 25.2	712.7 ± 34.3	690.8 ± 48.7	356.0 ± 20.7**	H	p ≤ 0.05
% Body Wgt	0.23 ± 0.01	0.21 ± 0.01**	0.22 ± 0.01	0.22 ± 0.02	0.12 ± 0.01**	NH	p ≤ 0.05
Thymus (mg)	585.8 ± 42.6	520.3 ± 35.3	535.5 ± 41.1	491.8 ± 39.1	87.4 ± 10.1**	H	NS
% Body Wgt	0.17 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.03 ± 0.01**	H	NS

Data obtained from page 193 of the study report
* $p \leq 0.05$; ** $p \leq 0.01$.
NS = not significant; H= homogenous; NH = non-homogeneous

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2. Histopathology: Did not perform.

E. IMMUNOTOXICITY TESTS:

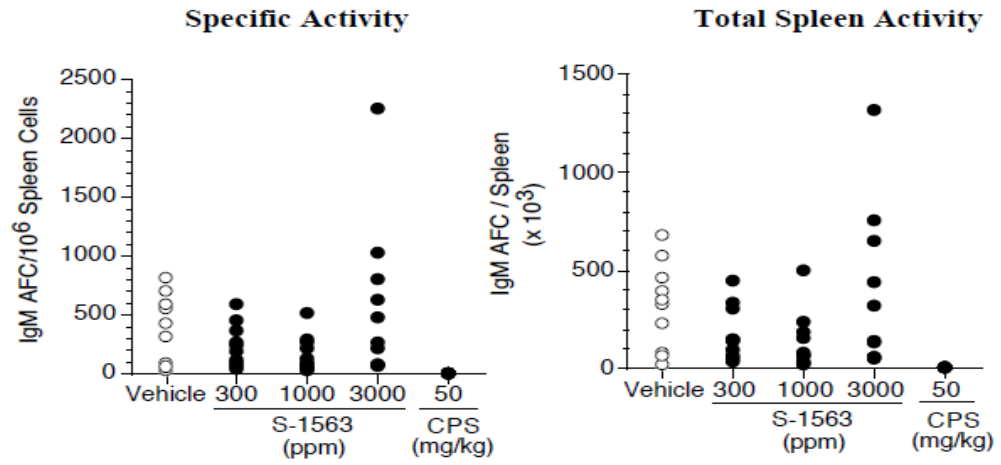
a. Antibody-Forming Cell (AFC) Assay: There were no statistically significant differences in the spleen AFC response, specific activity ($AFC/10^6$) as well as total spleen activity (AFC/spleen) in treated groups when compared to the vehicle control group. Lower AFC responses were observed at low and mid doses; however, no statistical significance and no dose-response relationship were observed. High inter-individual variability was noted in all the treatment groups as well as in the control group. Evaluation of the individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of TDAR to sheep RBC. The positive control group had statistically significantly ($p<0.01$) decreased AFC response (Table 5, Figure 1). This confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

Table 5. Antibody-Forming Cell Responses to T-dependent Sheep RBCs

Exposure	Body Wgt (g)	Spleen Wgt (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x\ 10^3$)
Vehicle Control	336 ± 4 (10)	769.4 ± 21.7 (10)	80.21 ± 4.96 (10)	414 ± 89 (10)	317 ± 70 (10)
S-1563					
300 ppm	335 ± 9 (10)	686.8 ± 25.2 (10)	74.99 ± 4.61 (10)	237 ± 57 (10)	175 ± 44 (10)
1000 ppm	322 ± 9 (10)	712.7 ± 34.3 (10)	73.65 ± 5.47 (10)	167 ± 48 (10)	134 ± 47 (10)
3000 ppm	311 ± 8 (10)	690.8 ± 48.7 (10)	68.24 ± 3.45 (10)	588 ± 213 (10)	390 ± 130 (10)
Cyclophosphamide 50 mg/kg	300 ± 8** (10)	356.0 ± 20.7** (10)	8.92 ± 0.36** (10)	0 ± 0** (10)	0 ± 0** (10)
H/NH	H	H	H	NH	NH
Trend Analysis	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	NS	NS

Data obtained from page 194 of study report
CP = Cyclophosphamide 50 mg/kg/day IP from Days 24-27

Figure 1. IgM Antibody-Forming Cell Responses to Sheep RBCs



Data obtained from page 190 of the study report.
CPS = Cyclophosphamide 50mg/kg/day IP from Days 24-27.

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b. <u>NK cell assay</u>: Did not perform NK cell assay.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: It was reported that the S-1563 did not reveal any signs of immunotoxicity when administered in the diet for 28 consecutive days to male Crl:WI(Han) rats. The NOAEL for immunotoxicity was 3000 ppm (equivalent to 241 mg/kg/day), the highest dose administered in this study.

B. REVIEWER COMMENTS: There were no premature deaths and no treatment-related clinical signs. There were no treatment- related effects on clinical observations, gross pathology, and organ weights. At 3000 ppm, there were treatment- related lower body weight gains and food consumption. The positive control group had decreased terminal body weights (11% less than vehicle control group) and statistically significantly ($p \leq 0.01$) decreased absolute and relative spleen and thymus weights.

The systemic LOAEL was 3000 ppm (equivalent to 241 mg/kg/day) based on lower body weight gains and food consumption. The systemic NOAEL was 1000 ppm (equivalent to 81 mg/kg/day).

There were no statistically significant differences in T-cell dependent antigen response (TDAR) to sheep red blood cells, either specific activity (AFC/10^6 spleen cells) or as total activity (AFC/spleen), between treated groups and the vehicle control group. Lower AFC responses were observed at low and mid doses; however, no statistical significance and no dose-response relationship were observed. High inter-individual variability was noted in all the treatment groups as well as in the control group. Evaluation of the individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of TDAR response to sheep RBCs. The positive control (CP) group had statistically significantly ($p \leq 0.01$) decreased TDAR response. This confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for S-1563 does not reveal any evidence of immunotoxicity. The overall weight of evidence suggests that the chemical does not directly target the immune system. Under HED guidance a NK cell activity assay is not required at this time.

The NOAEL for immunotoxicity was 3000 ppm (equivalent to 241 mg/kg/day), the highest dose tested. The immunotoxicity LOAEL was not established.

C. STUDY DEFICIENCIES: Minor deficiency: Stability data not provided.